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EUROPEAN PATENT APPLICATION

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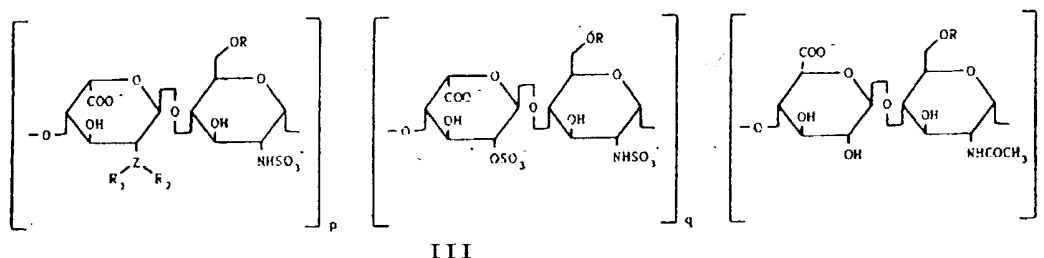
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Process for the synthesis of semisynthetic glycosaminoglycans with heparin or heparan structure modified in position 2 of the  $\alpha$ -L-iduronic-2-O-sulfate acid.

A process for the synthesis of semi-synthetic glycosaminoglycans of general formula III

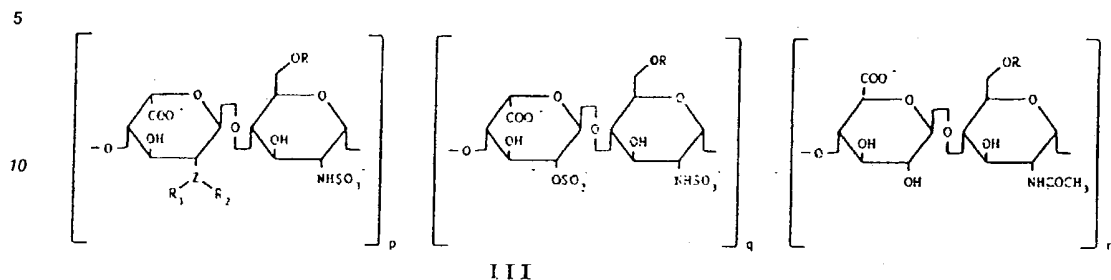


with heparin or heparan structure modified in position 2 of the  $\alpha$ -L-iduronic-2-O-sulfate acid in which the sulfate group is, entirely or in part, substituted with a nucleophilic radical is described. Said process is carried out by treating glycosaminoglycans with heparin or heparan structure by means of a nucleophilic agent in alkaline medium.

EP 0 618 234 A1

## SUMMARY OF THE INVENTION

A process for the synthesis of semi-synthetic glycosaminoglycans of general formula III



15 with heparin or heparan structure modified in position 2 of the  $\alpha$ -L-iduronic-2-O-sulfate acid in which the sulfate group is, entirely or in part, substituted with a nucleophilic radical is described. Said process is carried out by treating glycosaminoglycans with heparin or heparan structure by means of a nucleophilic agent in alkaline medium.

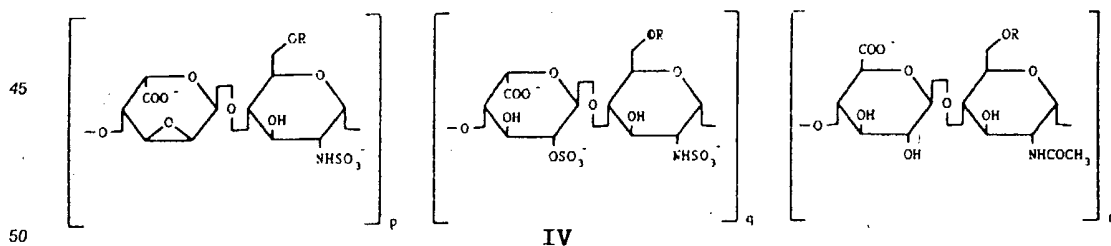
## BACKGROUND OF THE INVENTION

20 In the US patent 5,010,063 a description was given of a structural modification, in basic medium, of glycosaminoglycans with heparin and heparan structure with subsequent isolation from the reaction mixture of new derivatives with respect to the state of the art, as demonstrated unmistakably by the chemical and physical characteristics and especially by the  $^{13}\text{C}$ -NMR spectrum.

25 In the subsequent US patent 5,104,860 a further structural modification was described, in a basic or neutral medium, which, starting from the products formed in the reaction conditions described in US 5,010,063, and from the glycosaminoglycans with heparin or heparan structure used as starting products in the same patent, originated a range of new products, different from those described in said patent and new with respect to the state of the art, as demonstrated unmistakably by the chemical and physical characteristics and especially by the  $^{13}\text{C}$ -NMR spectrum.

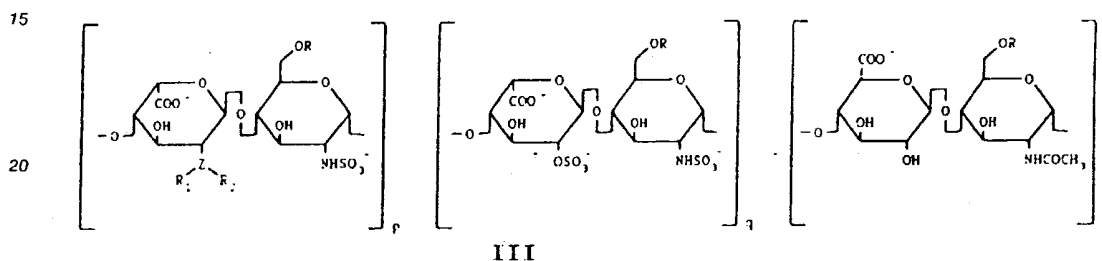
30 The chemical and physical characteristics of the products described in US patent 5,010,063 and the results of a subsequent structural study described by Jaseia M., Rej R., Sauriol F., Perlin A.S. in Can. J. Chem 67, 1449-56 (1989), with the specific aim of explaining the mechanism of the reaction of structural modification in a basic medium, have demonstrated that these derivatives show a modification which concerns just one of the monosaccharide units characteristic of glycosaminoglycans with heparin or heparan structure, more specifically the unit of  $\alpha$ -L-iduronic acid sulfated in position 2 and involving its transformation into a 2,3-poxygulonic unit. The so obtained epoxydes are represented from the following

40 general formula IV

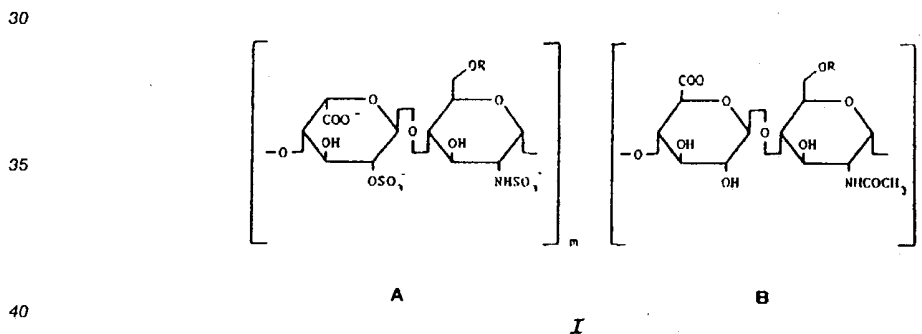


55 Likewise it has been demonstrated that semi-synthetic glycosaminoglycans with one 2,3-epoxygulonic unit and also glycosaminoglycans with heparin or heparan structure, in conditions of reaction similar to those described in US patent 5,104,860, undergo a structural modification which also concerns the saccharide unit of  $\alpha$ -L-iduronic acid sulfated in position 2 and involving the transformation of this saccharide unit into a unit of non-sulfated  $\alpha$ -L-iduronic acid or  $\alpha$ -L-galacturonic acid, according to the conditions of reaction used.

So US patent 5,010,063 describes semi-synthetic glycosaminoglycans containing an epoxy function between positions 2 and 3 of the unit of  $\alpha$ -L-iduronic-2-O-sulfate acid taken as a starting point and the conditions of reaction necessary for obtaining them, while US patent 5,104,860 describes products deriving from further transformation of the epoxyde, confirmed as having one unit of non-sulfated  $\alpha$ -L-iduronic or  $\alpha$ -L-galacturonic acid, and the conditions of reaction necessary for obtaining them starting from the epoxyde itself or, as an alternative, starting from the glycosaminoglycans with heparin or heparan structure themselves, used as starting products in US patent 5,010,063. Subsequently, in the published European patent application **EP 565.863** a description was given of semi-synthetic glycosaminoglycans in which one of the saccharide units characteristic of glycosaminoglycans with heparin or heparan structure, more specifically that containing  $\alpha$ -L-iduronic-2-O-sulfate acid, has undergone, totally or partly, a structural modification in position 2, position in which the sulfate group has been substituted with a nucleophilic radical. The process claimed in said published European patent application describes the obtaining of the semi-synthetic glycosaminoglycans of general formula III



by treating the epoxydes of formula IV, described in US patent 5,010,063 with a nucleophilic agent. Object of the present invention is a new process for the preparation of semi-synthetic glycosaminoglycans of general formula III directly starting from the glycosaminoglycans with heparin or heparan structure of general formula I



This new process represents an overcoming of the process described in the published European patent application **EP 565.863** because it uses as starting product the glycosaminoglycan of formula I while in said published European patent application the starting product was the epoxydic derivative of formula IV obtained in its turn from the glycosaminoglycan of formula I according to the process described in US patent 5,010,063. The advantage of directly obtaining the product of formula III in only one reaction by starting from the glycosaminoglycan of formula I instead of obtaining it by means of two consecutive reactions, the first of which includes the process of synthesis, isolation and purification of the epoxyde of formula IV starting from the glycosaminoglycan of formula I, is evident as regards the overall yield and the industrial cost.

To better define the field of the present invention, we would like to point out that the expression glycosaminoglycans with heparin or heparan structure is intended to indicate polysaccharides with a molecular weight of between about 3000 and about 50000 Daltons and characterized by the fact of possessing a disaccharide unit consisting of a uronic acid (which may be  $\alpha$ -L-iduronic or  $\beta$ -D-glucuronic) and of  $\alpha$ -D-glucosamine, connected, in alternate sequences, by 1,4-glycosidic bonds as described by Lindhal U., Kjellen L. in *Thrombosis and Haemostasis* 66, 44-48 (1991) and by Turnbull J.E., Gallagher J.T. in *Biochem. J.* 273, 553-559 (1991). Since the  $\alpha$ -L-iduronic acid can be sulfated in position 2 and the

5 to glycosaminoglycans with heparin or heparan structure



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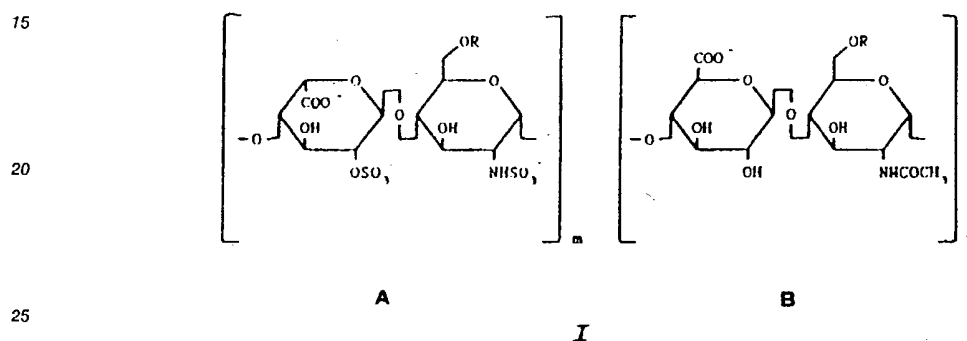
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compounds obtained in this way will be indicated as "semi-synthetic glycosaminoglycans with heparin or heparan structure of general formula IV in which  $-Z(R_2)R_1$  corresponds to .....

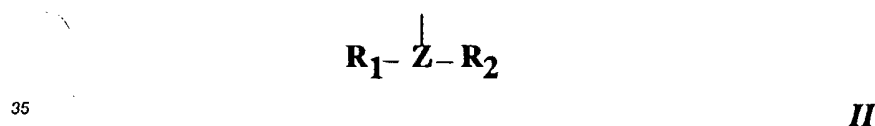
The reaction of structural modification which involves the partial or total introduction of the nucleophilic radical in position 2 of the  $\alpha$ -L-iduronic acid does not lead to the depolymerization of the glycosaminoglycans or alteration in the distribution of the molecular weight of the polysaccharide chains which form them, and for this reason the present reaction can be applied to glycosaminoglycans with heparin or heparan structure of any molecular weight. The products obtained can however be subjected to the known processes of chemical or enzymatic depolymerization.

## 10 DETAILED DESCRIPTION OF THE INVENTION

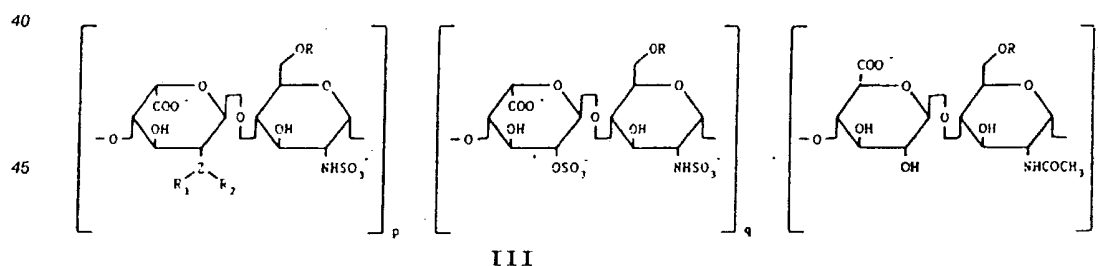
Object of the present invention is a new process for obtaining semi-synthetic glycosaminoglycans with heparin or heparan structure in which the original structure of general formula I



in which R represents hydrogen or the sulfate residue ( $\text{SO}_3^-$ ) and m and n are whole numbers with values between 1 and 100, has undergone a structural modification in position 2 of the  $\alpha$ -L-iduronic-2-O-sulfate acid with partial or total transformation of 2-O-sulfate group with a nucleophilic radical of general formula II



with formation of semi-synthetic glycosaminoglycans with heparin or heparan structure of general formula III



50 where  $p + q = m$ , with p other than 0, and m, n and R have the meaning defined above. All the nucleophilic reagents may be used to advantage in carrying out this invention and in fact the radical  $-Z(R_2)R_1$  includes any type of nucleophilic reagent.

55 More specifically, Z represents oxygen, sulphur or nitrogen,  $R_1$  represents the straight or branched ( $C_{1-12}$ ) alkyl, aminic, aromatic, diazo or hydroxyl radicals, substituted or not substituted and  $R_2$  represents null or hydrogen or a straight or branched ( $C_{1-6}$ ) alkyl radical, or taken with  $R_1$  forms a heterocyclic ring.

The radicals deriving from primary or secondary amines, secondary heterocyclical amines, amino-alcohols, aminothiols, amino acids, aminoesters, peptides, alcohols, phenols, mercaptans, dithiols,

thiophenols, hydroxylamines, hydrazines, hydrazides and sodium azide are preferred in performing the present invention.

Particularly preferable in performing this present invention are the radicals  $-Z(R_2)R_1$  originating from the following nucleophilic reagents: glycine, glycyglycine, L-cysteine, acetyl-L-cysteine, L-cysteine ethyl ester, 2-aminothiophenol, 1,3-propanedithiol, cysteamine, sodium azide, 2-aminoethyl bisulfate, taurine, thioglycolic acid,  $\beta$ -alanine ethyl ester, L-cystine, hydroxylamine, glycyLtaurine, cysteinyltaurine, glycyLcysteine, glycyLphenylalanine, glycyLtyrosine, 2-aminoethanol, glycine ester with 2-aminoethanol, glycine amide with 2-aminoethanol, arginyllysine, arginine, lysine, 2-aminoethanol ester with acetic acid, salicylic acid, methionine, glycyLproline,  $\gamma$ -aminobutyric acid, lysylprolylarginine, threonyllysylproline, threonyllysine, prolylarginine, lysylproline, choline, 4-(3-aminopropyl)-2-hydroxybenzoic acid and 4-(2-aminoethyl)-2-hydroxybenzoic acid. The process for obtaining semi-synthetic glycosaminoglycans with heparin or heparan structure of general formula III involves reacting a glycosaminoglycan with heparin or heparan structure of general formula I with a nucleophilic reagent whose radical is included in the general formula II, in aqueous solution and in the presence of a quantity of inorganic or organic base able to salify any acid groups present in the nucleophilic reagents and/or to free the same nucleophilic reagents from any salts they may have with substances of an acid nature and to create such an excess of alkalinity that the reaction mixture becomes from 0.01 N to 1 N with respect to the base used, preferably from 0.5 N to 1 N. The reaction is carried out by dissolving in water, at a concentration comprised between 1 % and 5 %, the glycosaminoglycan with heparin or heparan structure of formula I and by adding an aqueous solution containing the nucleophilic reagent and an inorganic or organic base to this solution. The quantity of nucleophilic agent is comprised between 1 and 200 molar equivalents, preferably between 10 and 100 molar equivalents, with respect to the dimeric unit of the glycosaminoglycan of formula I. Alkaline or alkaline-earth hydroxides, preferably sodium or potassium hydroxide, are used as inorganic bases, while tertiary amines like triethylamine are preferably used as organic bases.

The reaction mixture is kept under stirring, possibly in an atmosphere of inert gas, preferably nitrogen, where the nucleophilic reagent is easily oxidizable, at a temperature of between 0 °C and 50 °C, preferably between 10 °C and 40 °C, for a period of time of between 2 and 120 hours, preferably between 24 and 96 hours.

At the end of the reaction, after a possible cooling, the reaction mixture is given a neutral pH by adding an aqueous solution of hydrochloric acid. The excess of nucleophilic reagent may optionally be removed, for example through extraction with a solvent which is not miscible with water, with chloroform or diethyl ether, or through filtration where it is not soluble in aqueous medium with neutral pH. The clear aqueous solution may be further purified at a later stage through dialysis, cut off 3000 Daltons, first in running water and then in distilled water. Finally the semi-synthetic glycosaminoglycan with heparin or heparan structure of general formula III is isolated through lyophilization of the aqueous solution which contains it or through precipitation on addition of a suitable solvent.

The examples below are a further illustration of the invention but they must not be taken as a limitation of the invention itself.

#### EXAMPLE 1

**Semi-synthetic glycosaminoglycan with heparin structure of general formula III in which  $-Z(R_2)R_1$  corresponds to glycyL.**

A solution containing 3760 mg of glycine and 2500 mg of sodium hydroxide in 22,5 ml of water is added, at room temperature, to a solution containing in 2,5 ml of water 500 mg of heparin sodium salt.

The reaction mixture is kept under stirring at room temperature for 48 hours, then pH is neutralized through the addition of hydrochloric acid and the solution is then subjected to dialysis, cut off 3000 Daltons, for 12 hours in running water and for 6 hours in distilled water. The solution is then lyophilized and 480 mg of product are obtained.

#### EXAMPLE 2

**Semi-synthetic glycosaminoglycan with heparin structure of general formula III in which  $-Z(R_2)R_1$  corresponds to (S)-L-cysteinyl.**

The reaction is performed in the same conditions as described in example 1 using 8780 mg of L-cysteine monohydrate hydrochloride instead of 3760 mg of glycine and 4500 mg of sodium hydroxide

instead of 2500 mg.

520 Mg of product are obtained.

### EXAMPLE 3

**Semi-synthetic glycosaminoglycan with heparin structure of general formula III in which  $-Z(R_2)R_1$  corresponds to (S)-2-aminophenylthio.**

The reaction is performed in the same conditions as described in example 1 using 6300 mg of 2-aminothiophenol instead of 3760 mg of glycine and extending the reaction time to 72 hours. At the end of the reaction, after having neutralized the pH through the addition of hydrochloric acid, the excess of aminothiophenol is eliminated by extracting it with chloroform. The aqueous solution is then subjected to dialysis and lyophilization, as described in example 1, obtaining 530 mg of product.

### EXAMPLE 4

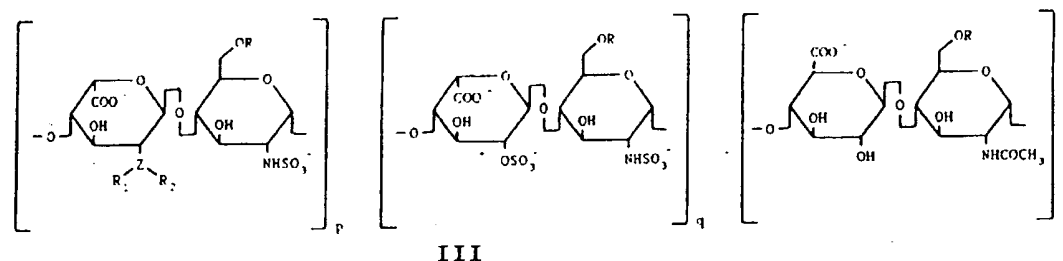
**Semi-synthetic glycosaminoglycan with heparin structure of general formula III in which  $-Z(R_2)R_1$  corresponds to (S)-carboxymethylthio.**

The reaction is performed in a nitrogen atmosphere in the same conditions as described in example 1 using 7416 mg of thioglycolic acid and 6900 mg of sodium hydroxide in 20 ml of water and extending the reaction time to 72 hours.

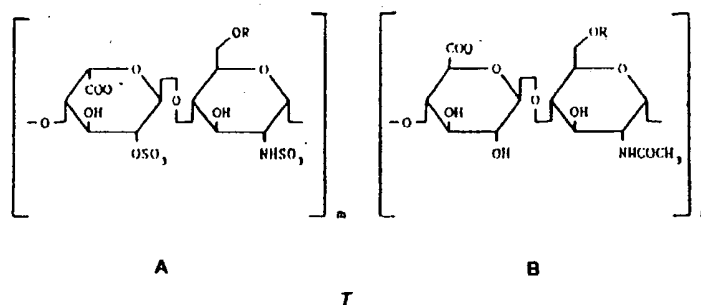
400 Mg of product are obtained.

### Claims

1. Process for the synthesis of semi-synthetic glycosaminoglycans with heparin or heparan structure of general formula III

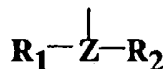


where  $p + q = m$ , with  $p$  other than 0, and  $m$  and  $n$  are whole numbers whose value is between 1 and 100,  $R$  represents hydrogen or the sulfate residue ( $SO_3^-$ ) and  $-Z(R_2)R_1$  represents a nucleophilic radical, which includes reacting a glycosaminoglycan with heparin or heparan structure of general formula I



with from 1 to 200 molar equivalents, with respect to the dimeric unit of the glycosaminoglycan with

heparin or heparan structure of general formula I, of a nucleophilic reagent whose radical is included within the general formula II



## II

in aqueous solution in the presence of a quantity of inorganic or organic base sufficient to salify any acid groups present in the nucleophilic reagents and/or to release the same nucleophilic reagents from any salts they may have with substances of an acid nature and to create such an excess of alkalinity that the reaction mixture becomes from 0.01 N to 1 N with respect to the base used, optionally in an atmosphere of inert gas, under stirring for a period of time between 2 and 120 hours at a temperature between 0°C and 50°C, neutralizing the pH of the cold aqueous solution through addition of an aqueous solution of hydrochloric acid, optionally removing the excess of nucleophilic reagent through extraction with a solvent not miscible with water or through filtration, subjecting the aqueous solution to dialysis with running water and with distilled water and isolating the product by means of lyophilization of the aqueous solution containing it or through precipitation by addition of a suitable solvent.

2. Process according to claim 1 characterized by the fact that the quantity of nucleophilic agent is between 10 and 100 molar equivalents with respect to the dimeric unit of the glycosaminoglycan of general formula I and that the concentration of said glycosaminoglycan in aqueous solution is comprised between 1% and 5%
3. Process according to claim 1 characterized by the fact that the base used is selected from sodium hydroxide, potassium hydroxide and triethylamine and that the excess of alkalinity is such that the reaction mixture becomes from 0.5 N to 1 N with respect to the base used.
4. Process according to each of previous claims characterized by the fact that Z represents oxygen, sulphur or nitrogen, R<sub>1</sub> represents the straight or branched (C<sub>1-12</sub>) alkyl, aminic, aromatic, diazoic or hydroxyl radicals, substituted or not substituted and R<sub>2</sub> represents null or a straight or branched (C<sub>1-6</sub>) alkyl radical, or taken with R<sub>1</sub> forms a heterocyclic ring.
5. Process according to claim 4 characterized by the fact that the radical -Z(R<sub>2</sub>)R<sub>1</sub> derives from primary or secondary amines, secondary heterocyclic amines, aminoalcohols, aminothiols, aminoacids, aminoesters, peptides, alcohols, phenols, mercaptans, dithiols, thiophenols, hydroxylamines, hydrazines, hydrazides and sodium azide.
6. Process according to claim 5 characterized by the fact that the radical -Z(R<sub>2</sub>)R<sub>1</sub> derives from glycine, glycyglycine, L-cysteine, acetyl-L-cysteine, L-cysteine ethyl ester, 2-aminothiophenol, 1,3-propandithiol, cysteamine, sodium azide, 2-aminoethyl bisulfate, taurine, thioglycolic acid, β-alanine ethyl ester, L-cystine, hydroxylamine, glycytaurine, cysteinyltaurine, glycyrcysteine, glycyphenylalanine, glycytyrosine, 2-aminoethanol, glycine ester with 2-aminoethanol, glycine amide with 2-aminoethanol, arginyllysine, arginine, lysine, 2-aminoethanol ester with acetic acid, salicylic acid, methionine, glycyproline, γ-aminobutyric acid, lysylprolylarginine, threonyllysylproline, threonyllysine, prolylarginine, lysylproline, choline, 4-(3-aminopropyl)-2-hydroxybenzoic acid and 4-(2-aminoethyl)-2-hydroxybenzoic acid.





European Patent  
Office

## EUROPEAN SEARCH REPORT

Application Number  
EP 94 10 4306

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 5)
A	EP-A-0 485 748 (ALFA WASSERMAN) ---		C08B37/10
A	WO-A-92 20349 (GENZYME CORPORATION) ---		
A	US-A-5 011 919 (FUSSI ET AL.) ---		
D, P, X	EP-A-0 565 863 (ALFA WASSERMAN) 20 October 1993 ---	1	
E	EP-A-0 598 474 (SYNTEX) 25 May 1994 * page 3, line 20 - line 25 * * page 4; example 1 * -----	1	
			TECHNICAL FIELDS SEARCHED (Int. Cl. 5)
			C08B
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 12 July 1994	Examiner Lensen, H
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ----- A : member of the same patent family, corresponding document	

(19)



Europäisches Patentamt  
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(11)

**EP 0 618 234 B1**

(12)

**EUROPEAN PATENT SPECIFICATION**

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(51) Int Cl.<sup>6</sup>: **C08B 37/10**

(21) Application number: **94104306.9**

(22) Date of filing: **18.03.1994**

(54) **Process for the synthesis of semisynthetic glycosaminoglycans with heparin or heparan structure modified in position 2 of the alpha-L-iduronic-2-O-sulfate acid**

Verfahren zur Synthese von halbsynthetischen Glykosaminoglykanen mit Heparin- oder Heparanstruktur, die in 2-Position modifiziertes Alpha-L-Iduronsäure-2-O-Sulfat enthalten

Procédé de synthèse de glycosaminoglycans semi-synthétiques possédant le motif acide alpha L-iduronique-2-O-sulfate de l'héparine ou de l'héparane modifié en position 2

(84) Designated Contracting States:

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**80539 München (DE)**

(73) Proprietor: **ALFA WASSERMANN S.p.A.**  
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(56) References cited:

**EP-A- 0 485 748**

**EP-A- 0 565 863**

**EP-A- 0 598 474**

**WO-A-92/20349**

**US-A- 5 011 919**

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**I-40134 Bologna (IT)**

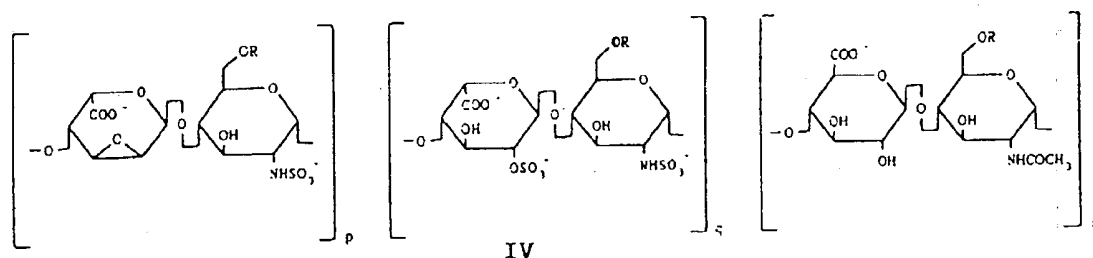
Note: Within nine months from the publication of the mention of the grant of the European patent, any person may give notice to the European Patent Office of opposition to the European patent granted. Notice of opposition shall be filed in a written reasoned statement. It shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European Patent Convention).

## Description

In the US patent 5,010,063 a description was given of a structural modification, in basic medium, of glycosaminoglycans with heparin and heparan structure with subsequent isolation from the reaction mixture of new derivatives with respect to the state of the art, as demonstrated unmistakably by the chemical and physical characteristics and especially by the  $^{13}\text{C}$ -NMR spectrum.

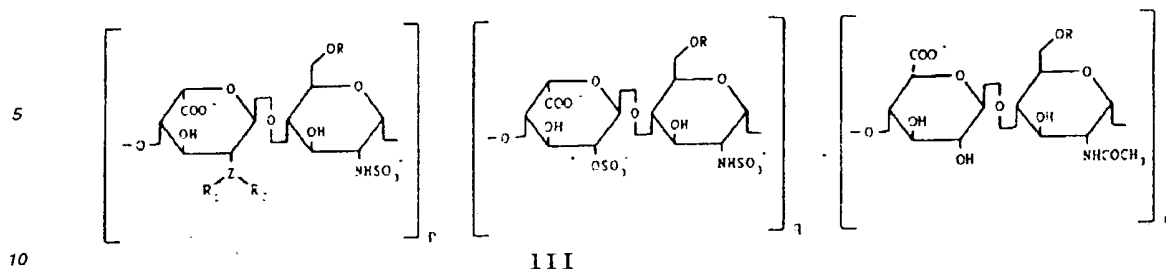
In the subsequent US patent 5,104,860 a further structural modification was described, in a basic or neutral medium, which, starting from the products formed in the reaction conditions described in US 5,010,063, and from the glycosaminoglycans with heparin or heparan structure used as starting products in the same patent, originated a range of new products, different from those described in said patent and new with respect to the state of the art, as demonstrated unmistakably by the chemical and physical characteristics and especially by the  $^{13}\text{C}$ -NMR spectrum.

The chemical and physical characteristics of the products described in US patent 5,010,063 and the results of a subsequent structural study described by Jaseia M., Rej R., Sauriol F., Perlin A.S. in Can. J. Chem 67, 1449-56 (1989), with the specific aim of explaining the mechanism of the reaction of structural modification in a basic medium, have demonstrated that these derivatives show a modification which concerns just one of the monosaccharide units characteristic of glycosaminoglycans with heparin or heparan structure, more specifically the unit of  $\alpha$ -L-iduronic acid sulfated in position 2 and involving its transformation into a 2,3-epoxygulonic unit. The so obtained epoxydes are represented from the following general formula IV



Likewise it has been demonstrated that semi-synthetic glycosaminoglycans with one 2,3-epoxygulonic unit and also glycosaminoglycans with heparin or heparan structure, in conditions of reaction similar to those described in US patent 5,104,860, undergo a structural modification which also concerns the saccharide unit of  $\alpha$ -L-iduronic acid sulfated in position 2 and involving the transformation of this saccharide unit into a unit of non-sulfated  $\alpha$ -L-iduronic acid or  $\alpha$ -L-galacturonic acid, according to the conditions of reaction used.

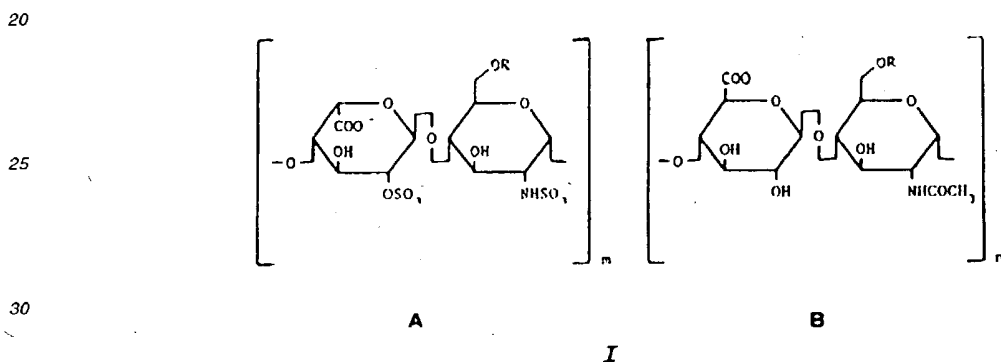
So US patent 5,010,063 describes semi-synthetic glycosaminoglycans containing an epoxy function between positions 2 and 3 of the unit of  $\alpha$ -L-iduronic-2-O-sulfate acid taken as a starting point and the conditions of reaction necessary for obtaining them, while US patent 5,104,860 describes products deriving from further transformation of the epoxyde, confirmed as having one unit of non-sulfated  $\alpha$ -L-iduronic or  $\alpha$ -L-galacturonic acid, and the conditions of reaction necessary for obtaining them starting from the epoxyde itself or, as an alternative, starting from the glycosaminoglycans with heparin or heparan structure themselves, used as starting products in US patent 5,010,063. Subsequently, in the published European patent application **EP 565.863** a description was given of semi-synthetic glycosaminoglycans in which one of the saccharide units characteristic of glycosaminoglycans with heparin or heparan structure, more specifically that containing  $\alpha$ -L-iduronic-2-O-sulfate acid, has undergone, totally or partly, a structural modification in position 2, position in which the sulfate group has been substituted with a nucleophilic radical. The process claimed in said published European patent application describes the obtaining of the semi-synthetic glycosaminoglycans of general formula III



by treating the epoxydes of formula IV, described in US patent 5,010,063 with a nucleophilic agent.

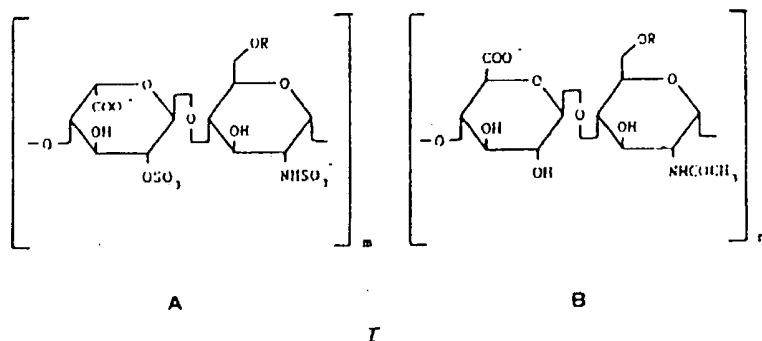
EP-A-0 598 474 discloses a method for the production of 2-Deoxy-2-amino hexuronyl derivatives of heparin. This method comprises heating the starting material, i.e. heparin and a solution of concentrated ammonium hydroxide in a sealed reactor under pressure at a temperature of 60 to 80°C for a period of 3 to 4 hours.

Object of the present invention is a new process for the preparation of semi-synthetic glycosaminoglycans of general formula III directly starting from the glycosaminoglycans with heparin or heparan structure of general formula I



This new process represents an overcoming of the process described in the published European patent application EP 565.863 because it uses as starting product the glycosaminoglycan of formula I while in said published European patent application the starting product was the epoxydic derivative of formula IV obtained in its turn from the glycosaminoglycan of formula I according to the process described in US patent 5,010,063. The advantage of directly obtaining the product of formula III in only one reaction by starting from the glycosaminoglycan of formula I instead of obtaining it by means of two consecutive reactions, the first of which includes the process of synthesis, isolation and purification of the epoxyde of formula IV starting from the glycosaminoglycan of formula I, is evident as regards the overall yield and the industrial cost.

To better define the field of the present invention, we would like to point out that the expression glycosaminoglycans with heparin or heparan structure is intended to indicate polysaccharides with a molecular weight of between about 3000 and about 50000 Daltons and characterized by the fact of possessing a disaccharide unit consisting of a uronic acid (which may be  $\alpha$ -L-iduronic or  $\beta$ -D-glucuronic) and of  $\alpha$ -D-glucosamine, connected, in alternate sequences, by 1,4-glycosidic bonds as described by Lindhal U., Kjellen L. in *Thrombosis and Haemostasis* 66, 44-48 (1991) and by Turnbull J.E., Gallagher J.T. in *Biochem. J.* 273, 553-559 (1991). Since the  $\alpha$ -L-iduronic acid can be sulfated in position 2 and the glucosamine can be N-acetylated, N-sulfated, 6-O-sulfated, 3-O-sulfated, according to the variable positions of the substituents, at least 10 different disaccharide units are possible, whose combination may generate a large number of different sequences. Bearing in mind the most represented disaccharide units and the most frequent sequences, we can say with reasonable approximation, that the general formula I can be attributed to glycosaminoglycans with heparin or heparan structure



where R represents hydrogen or the sulfate residue ( $\text{SO}_3^-$ ) and where m and n are whole numbers between 1 and 100.

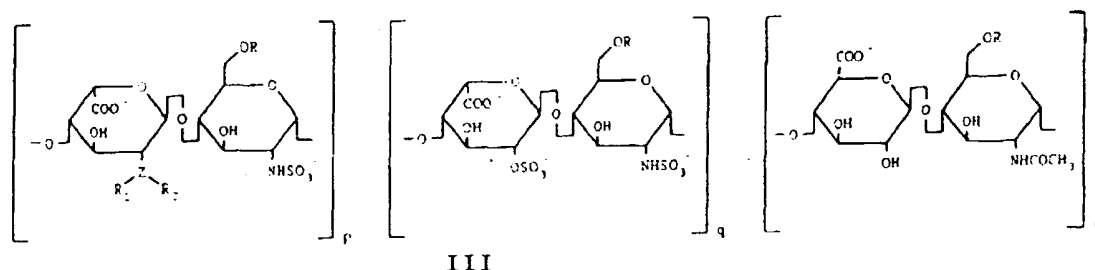
In heparin structured glycosaminoglycans of natural origin the value of m is high and the disaccharide unit A represents about 80% of the disaccharide units; on the contrary, in heparan structured glycosaminoglycans of natural origin the value of n is high and the disaccharide unit B represents about 80% of the disaccharide units.

The general formulae I and III are intended to reveal the composition of the main saccharide units but make no reference to their sequence.

As is known to experts in the art, it is possible to make a chemical modification of glycosaminoglycans of natural origin, for example through reactions of N-desulfation, possibly followed by reactions of N-acetylation, thus also obtaining semi-synthetic N-desulfated heparins or N-desulfated-N-acetylated heparins. In addition, these glycosaminoglycans, whether natural or semi-synthetic, may be subjected to depolymerization processes by means of which the molecular weight is taken to levels generally between 3000 and 10000 Daltons.

The structural modification described in this invention for obtaining new semi-synthetic glycosaminoglycans with heparin or heparan structure refers to the unit of  $\alpha$ -L-iduronic-2-O-sulfate acid where the partial or total selective substitution of the O-sulfate group in position 2 with a nucleophilic residue, whatever it might be the desired compound with heparin or heparan structure, takes place. Indeed, besides being selective, the chemical process described in this invention can be applied to glycosaminoglycans with heparin or heparan structure which present all the possible sequences; ie. it is independent of the type and of the level of functionalization of the saccharide unit which precedes or follows in the sequence the unit of  $\alpha$ -L-iduronic-2-O-sulfate acid which is the object of the reaction of structural modification.

The structure of the new products is represented by the general formula III

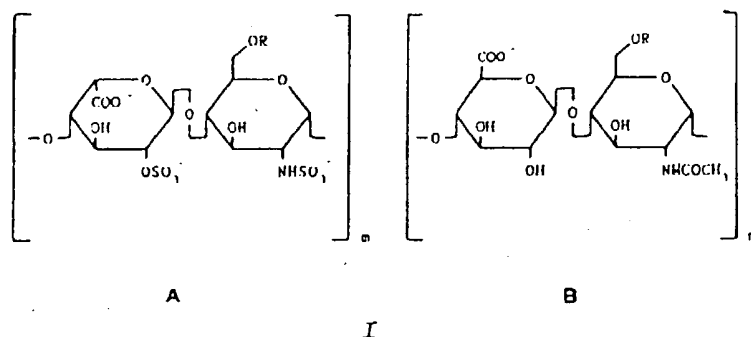


where  $p + q = m$ , with p other than 0, and m, n and R have the meaning as seen above, and where  $-Z(R_2)R_1$  represents the nucleophilic group introduced through the process described in this invention. The compounds obtained in this way will be indicated as "semi-synthetic glycosaminoglycans with heparin or heparan structure of general formula III in which  $-Z(R_2)R_1$  corresponds to a nucleophilic radical, wherein Z represents oxygen, sulphur or nitrogen,  $R_1$  represents the straight or branched ( $\text{C}_{1-12}$ ) alkyl, aminic, aromatic, diazoic or hydroxyl radicals, substituted or not substituted and  $R_2$  represents null, hydrogen or a straight or branched ( $\text{C}_{1-6}$ ) alkyl radical, or taken with  $R_1$  forms a heterocyclic ring".

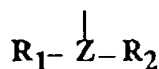
The reaction of structural modification which involves the partial or total introduction of the nucleophilic radical in position 2 of the  $\alpha$ -L-iduronic acid does not lead to the depolymerization of the glycosaminoglycans or alteration in the distribution of the molecular weight of the polysaccharide chains which form them, and for this reason the present reaction can be applied to glycosaminoglycans with heparin or heparan structure of any molecular weight. The products obtained can however be subjected to the known processes of chemical or enzymatic depolymerization.

**DETAILED DESCRIPTION OF THE INVENTION**

Object of the present invention is a new process for obtaining semi-synthetic glycosaminoglycans with heparin or heparan structure in which the original structure of general formula I

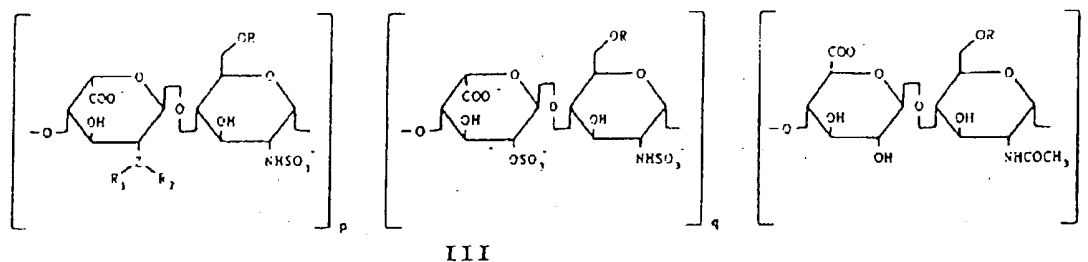


in which R represents hydrogen or the sulfate residue ( $\text{SO}_3^-$ ) and m and n are whole numbers with values between 1 and 100, has undergone a structural modification in position 2 of the  $\alpha$ -L-iduronic-2-O-sulfate acid with partial or total transformation of 2-O-sulfate group with a nucleophilic radical of general formula II



II

with formation of semi-synthetic glycosaminoglycans with heparin or heparan structure of general formula III



where  $p + q = m$  with p other than 0, and m, n and R have the meaning defined above. All the nucleophilic reagents may be used to advantage in carrying out this invention and in fact the radical  $-\text{Z}(\text{R}_2)\text{R}_1$  includes any type of nucleophilic reagent.

More specifically, Z represents oxygen, sulphur or nitrogen,  $\text{R}_1$  represents the straight or branched ( $\text{C}_{1-12}$ ) alkyl, aminic, aromatic, diazo or hydroxyl radicals, substituted or not substituted and  $\text{R}_2$  represents null or hydrogen or a straight or branched ( $\text{C}_{1-6}$ ) alkyl radical, or taken with  $\text{R}_1$  forms a heterocyclic ring.

The radicals deriving from primary or secondary amines, secondary heterocyclical amines, amino-alcohols, aminothiols, amino acids, aminoesters, peptides, alcohols, phenols, mercaptans, dithiols, thiophenols, hydroxylamines, hydrazines, hydrazides and sodium azide are preferred in performing the present invention.

Particularly preferable in performing this present invention are the radicals  $-\text{Z}(\text{R}_2)\text{R}_1$  originating from the following nucleophilic reagents: glycine, glycyglycine, L-cysteine, acetyl-L-cysteine, L-cysteine ethyl ester, 2-aminothiophenol, 1,3-propandithiol, cysteamine, sodium azide, 2-aminoethyl bisulfate, taurine, thioglycolic acid,  $\beta$ -alanine ethyl ester, L-cystine, hydroxylamine, glycyllaurine, cysteinyltaurine, glycylycysteine, glycylyphenylalanine, glycylytyrosine, 2-aminoethanol, glycine ester with 2-aminoethanol, glycine amide with 2-aminoethanol, arginyllysine, arginine, lysine, 2-aminoeth-

anol ester with acetic acid, salicylic acid, methionine, glycylproline,  $\gamma$ -aminobutyric acid, lysylprolylarginine, threonyl-lysylproline, threonyllysine, prolylarginine, lysylproline, choline, 4-(3-aminopropyl)-2-hydroxybenzoic acid and 4-(2-aminoethyl)-2-hydroxybenzoic acid.

The process for obtaining semi-synthetic glycosaminoglycans with heparin or heparan structure of general formula III involves reacting a glycosaminoglycan with heparin or heparan structure of general formula I with a nucleophilic reagent whose radical is included in the general formula II, in aqueous solution and in the presence of a quantity of inorganic or organic base able to salify any acid groups present in the nucleophilic reagents and/or to free the same nucleophilic reagents from any salts they may have with substances of an acid nature and to create such an excess of alkalinity that the reaction mixture becomes from 0.01 N to 1 N with respect to the base used, preferably from 0.5 N to 1 N. The reaction is carried out by dissolving in water, at a concentration comprised between 1% and 5%, the glycosaminoglycan with heparin or heparan structure of formula I and by adding an aqueous solution containing the nucleophilic reagent and an inorganic or organic base to this solution. The quantity of nucleophilic agent is comprised between 1 and 200 molar equivalents, preferably between 10 and 100 molar equivalents, with respect to the dimeric unit of the glycosaminoglycan of formula I.

Alkaline or alkaline-earth hydroxides, preferably sodium or potassium hydroxide, are used as inorganic bases, while tertiary amines like triethylamine are preferably used as organic bases.

The reaction mixture is kept under stirring, possibly in an atmosphere of inert gas, preferably nitrogen, where the nucleophilic reagent is easily oxidizable, at a temperature of between 10°C and 40°C, for a period of time of between 24 and 96 hours.

At the end of the reaction, after a possible cooling, the reaction mixture is given a neutral pH by adding an aqueous solution of hydrochloric acid. The excess of nucleophilic reagent may optionally be removed, for example through extraction with a solvent which is not miscible with water, with chloroform or diethyl ether, or through filtration where it is not soluble in aqueous medium with neutral pH. The clear aqueous solution may be further purified at a later stage through dialysis, cut off 3000 Daltons, first in running water and then in distilled water. Finally the semi-synthetic glycosaminoglycan with heparin or heparan structure of general formula III is isolated through lyophilization of the aqueous solution which contains it or through precipitation on addition of a suitable solvent.

The examples below are a further illustration of the invention but they must not be taken as a limitation of the invention itself.

#### EXAMPLE 1

##### Semi-synthetic glycosaminoglycan with heparin structure of general formula III in which $-Z(R_2)R_1$ corresponds to glycyl.

A solution containing 3760 mg of glycine and 2500 mg of sodium hydroxide in 22,5 ml of water is added, at room temperature, to a solution containing in 2,5 ml of water 500 mg of heparin sodium salt.

The reaction mixture is kept under stirring at room temperature for 48 hours, then pH is neutralized through the addition of hydrochloric acid and the solution is then subjected to dialysis, cut off 3000 Daltons, for 12 hours in running water and for 6 hours in distilled water. The solution is then lyophilized and 480 mg of product are obtained.

#### EXAMPLE 2

##### Semi-synthetic glycosaminoglycan with heparin structure of general formula III in which $-Z(R_2)R_1$ corresponds to (S)-L-cysteinyl.

The reaction is performed in the same conditions as described in example 1 using 8780 mg of L-cysteine monohydrate hydrochloride instead of 3760 mg of glycine and 4500 mg of sodium hydroxide instead of 2500 mg.

520 Mg of product are obtained.

#### EXAMPLE 3

##### Semi-synthetic glycosaminoglycan with heparin structure of general formula III in which $-Z(R_2)R_1$ corresponds to (S)-2-aminophenylthio.

The reaction is performed in the same conditions as described in example 1 using 6300 mg of 2-aminothiophenol instead of 3760 mg of glycine and extending the reaction time to 72 hours. At the end of the reaction, after having neutralized the pH through the addition of hydrochloric acid, the excess of aminothiophenol is eliminated by extracting it with chloroform. The aqueous solution is then subjected to dialysis and lyophilization, as described in example 1,

obtaining 530 mg of product.

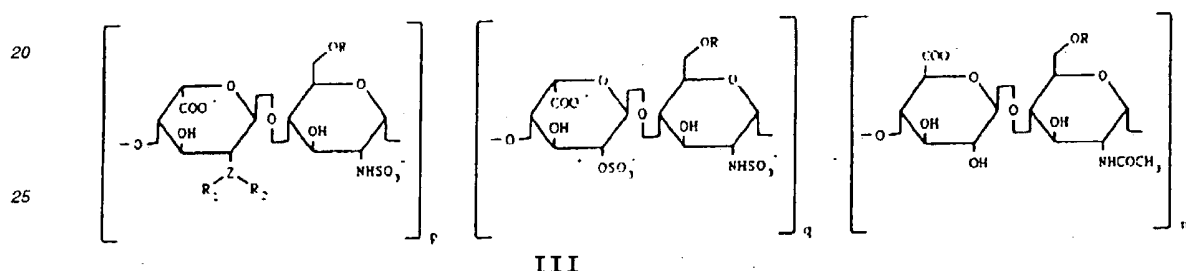
#### EXAMPLE 4

- 5 Semi-synthetic glycosaminoglycan with heparin structure of general formula III in which  $-Z(R_2)R_1$  corresponds to (S)-carboxymethylthio.

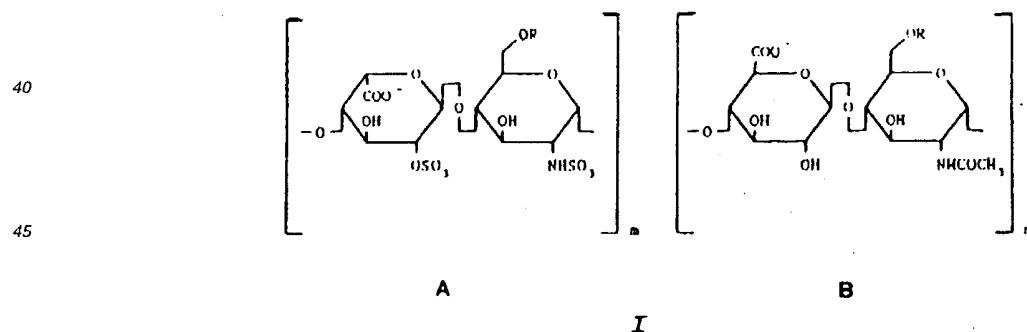
The reaction is performed in a nitrogen atmosphere in the same conditions as described in example 1 using 7416 mg of thioglycolic acid and 6900 mg of sodium hydroxide in 20 ml of water and extending the reaction time to 72 hours.  
10 400 Mg of product are obtained.

#### Claims

- 15 1. Process for the synthesis of semi-synthetic glycosaminoglycans with heparin or heparan structure of general formula III



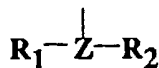
- 30 where  $p + q = m$ , with  $p$  other than 0, and  $m$  and  $n$  are whole numbers whose value is between 1 and 100,  $R$  represents hydrogen or the sulfate residue ( $SO_3^-$ ) and  $-Z(R_2)R_1$  represents a nucleophilic radical, wherein  $Z$  represents oxygen, sulphur or nitrogen,  $R_1$  represents the straight or branched ( $C_{1-12}$ ) alkyl, aminic, aromatic, diazoic or hydroxyl radicals, substituted or not substituted and  $R_2$  represents null, hydrogen or a straight or branched ( $C_{1-6}$ ) alkyl radical, or taken with  $R_1$  forms a heterocyclic ring, which includes reacting a glycosaminoglycan with heparin or heparan structure of general formula I
- 35



50 with from 1 to 200 molar equivalents, with respect to the dimeric unit of the glycosaminoglycan with heparin or heparan structure of general formula I, of a nucleophilic reagent whose radical is included within the general formula II

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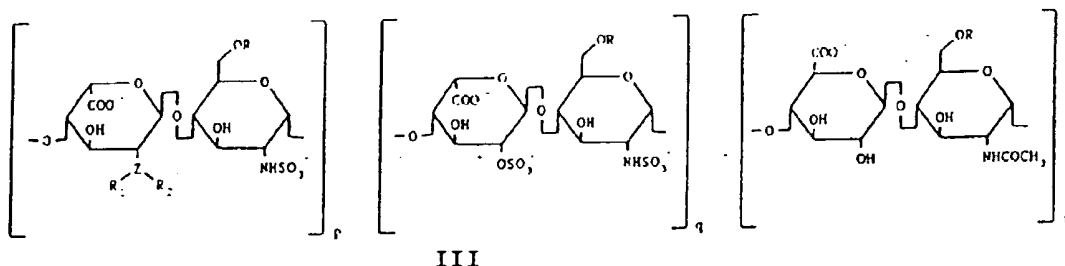
## II

in aqueous solution in the presence of a quantity of inorganic or organic base sufficient to salify any acid groups present in the nucleophilic reagents and/or to release the same nucleophilic reagents from any salts they may have with substances of an acid nature and to create such an excess of alkalinity that the reaction mixture becomes from 0.01 N to 1 N with respect to the base used, optionally in an atmosphere of inert gas, under stirring for a period of time between 24 and 96 hours at a temperature between 10°C and 40°C neutralizing the pH of the cold aqueous solution through addition of an aqueous solution of hydrochloric acid, optionally removing the excess of nucleophilic reagent through extraction with a solvent not miscible with water or through filtration, subjecting the aqueous solution to dialysis with running water and with distilled water and isolating the product by means of lyophilization of the aqueous solution containing it or through precipitation by addition of a suitable solvent.

2. Process according to claim 1 characterized by the fact that the quantity of nucleophilic agent is between 10 and 100 molar equivalents with respect to the dimeric unit of the glycosaminoglycan of general formula I and that the concentration of said glycosaminoglycan in aqueous solution is comprised between 1% and 5%
3. Process according to claim 1 characterized by the fact that the base used is selected from sodium hydroxide, potassium hydroxide and triethylamine and that the excess of alkalinity is such that the reaction mixture becomes from 0.5 N to 1 N with respect to the base used.
4. Process according to claim 1 characterized by the fact that the radical  $-\text{Z}(\text{R}_2)\text{R}_1$  derives from primary or secondary amines, secondary heterocyclic amines, aminoalcohols, aminothiols, aminoacids, aminoesters, peptides, alcohols, phenols, mercaptans, dithiols, thiophenols, hydroxylamines, hydrazines, hydrazides and sodium azide.
5. Process according to claim 4 characterized by the fact that the radical  $-\text{Z}(\text{R}_2)\text{R}_1$  derives from glycine, glycyglycine, L-cysteine, acetyl-L-cysteine, L-cysteine ethyl ester, 2-aminothiophenol, 1,3-propandithiol, cysteamine, sodium azide, 2-aminoethyl bisulfate, taurine, thioglycolic acid,  $\beta$ -alanine ethyl ester, L-cystine, hydroxylamine, glycytaurine, cysteinyltaurine, glycyrcysteine, glycyphenylalanine, glycytyrosine, 2-aminoethanol, glycine ester with 2-aminoethanol, glycine amide with 2-aminoethanol, arginyllysine, arginine, lysine, 2-aminoethanol ester with acetic acid, salicylic acid, methionine, glycyproline,  $\gamma$ -aminobutyric acid, lysylprolylarginine, threonyllysylproline, threonyllysine, prolylarginine, lysylproline, choline, 4-(3-aminopropyl)-2-hydroxybenzoic acid and 4-(2-aminoethyl)-2-hydroxybenzoic acid.

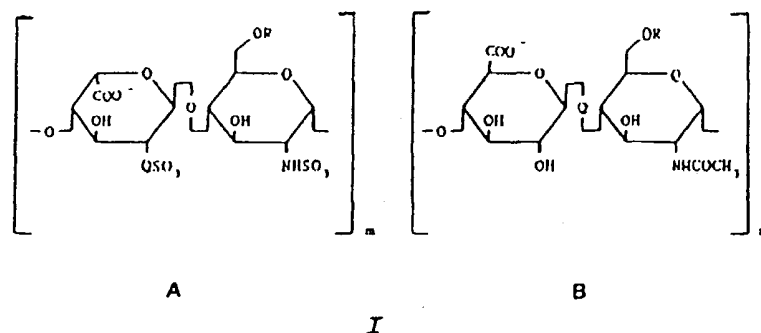
## Patentansprüche

1. Verfahren zur Synthese semisynthetischer Glycosaminoglycane mit Heparin- oder Heparanstruktur der allgemeinen Formel III

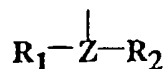


worin  $p + q = m$  bedeuten, wobei  $p$  einen anderen Wert als 0 besitzt, und  $m$  und  $n$  ganze Zahlen bedeuten, deren Wert zwischen 1 und 100 liegt,  $R$  Wasserstoff oder den Sulfatrest ( $\text{SO}_3^-$ ) bedeutet und  $-\text{Z}(\text{R}_2)\text{R}_1$  eine nukleophile

Gruppe bedeutet, worin Z Sauerstoff, Schwefel oder Stickstoff bedeutet,  $R_1$  geradkettiges oder verzweigt-kettiges ( $C_{1-12}$ )-Alkyl, eine Amino-, aromatische, Diazo- oder Hydroxylgruppe, substituiert oder nichtsubstituiert, bedeutet und  $R_2$  nicht vorhanden ist, Wasserstoff oder eine geradkettige oder verzweigt-kettige ( $C_{1-6}$ )-Alkylgruppe bedeutet oder zusammen mit  $R_1$  einen heterocyclischen Ring bildet, umfassend die Umsetzung eines Glycosaminoglycans mit einer Heparin- oder Heparanstruktur der allgemeinen Formel I



mit 1 bis 200 Moläquivalenten, bezogen auf die dimere Einheit des Glycosaminoglycans mit Heparin- oder Heparanstruktur der allgemeinen Formel I, eines nukleophilen Reagenses, dessen Gruppe die allgemeine Formel II



II

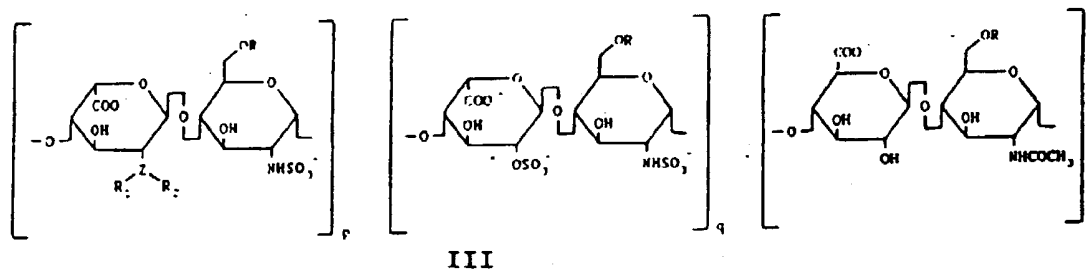
besitzt, in wäßriger Lösung in Anwesenheit einer Menge an einer anorganischen oder organischen Base, die ausreicht, irgendwelche sauren Gruppen, die in den nukleophilen Reagentien vorhanden sind, in Salzform zu überführen und/oder die gleichen nukleophilen Reagentien aus irgendwelchen Salzen, die mit Substanzen mit saurer Natur gebildet worden sind, freizusetzen und einen solchen Überschuß an Alkalinität zu ergeben, daß das Reaktionsgemisch 0,01 N bis 1 N, bezogen auf die verwendete Base, wird, gegebenenfalls in einer Inertgasatmosphäre, unter Rühren während einer Zeit zwischen 24 und 96 Stunden bei einer Temperatur zwischen 10°C und 40°C, Neutralisation des pH der kalten wäßrigen Lösung durch Zugabe einer wäßrigen Lösung an Chlorwasserstoffsäure, gegebenenfalls Entfernung des Überschusses an nukleophilem Reagens durch Extraktion mit einem mit Wasser nicht mischbaren Lösungsmittel oder durch Filtration, Durchführung einer Dialyse mit der wäßrigen Lösung mit fließendem Wasser und mit destilliertem Wasser und Isolierung des Produkts durch Lyophilisierung der wäßrigen Lösung, die es enthält, oder durch Präzipitation durch Zugabe eines geeigneten Lösungsmittels.

2. Verfahren nach Anspruch 1, dadurch **gekennzeichnet**, daß die Menge an nukleophilem Mittel zwischen 10 und 100 Moläquivalenten, bezogen auf die dimere Einheit des Glycosaminoglycans der allgemeinen Formel I, beträgt und daß die Konzentration an Glycosaminoglycan in der wäßrigen Lösung zwischen 1% und 5% liegt.
3. Verfahren nach Anspruch 1, dadurch **gekennzeichnet**, daß die verwendete Base ausgewählt wird aus Natriumhydroxid, Kaliumhydroxid und Triethylamin, und daß der Überschuß der Alkalinität so ist, daß das Reaktionsgemisch 0,5 N bis 1 N, bezogen auf die verwendete Base, ist.
4. Verfahren nach Anspruch 1, dadurch **gekennzeichnet**, daß sich die Gruppe  $-Z(R_2)R_1$  von primären oder sekundären Aminen, sekundären heterocyclischen Aminen, Aminoalkoholen, Aminothiolen, Aminosäuren, Aminoestern, Peptiden, Alkoholen, Phenolen, Mercaptanen, Dithiolen, Thiophenolen, Hydroxylaminen, Hydrazinen, Hydraziden und Natriumazid ableitet.
5. Verfahren nach Anspruch 4, dadurch **gekennzeichnet**, daß sich die Gruppe  $-Z(R_2)R_1$  von Glycin, Glycylglycin, L-Cystein, Acetyl-L-cystein, L-Cysteinethylester, 2-Aminothiophenol, 1,3-Propandithiol, Cysteamin, Natriumazid,

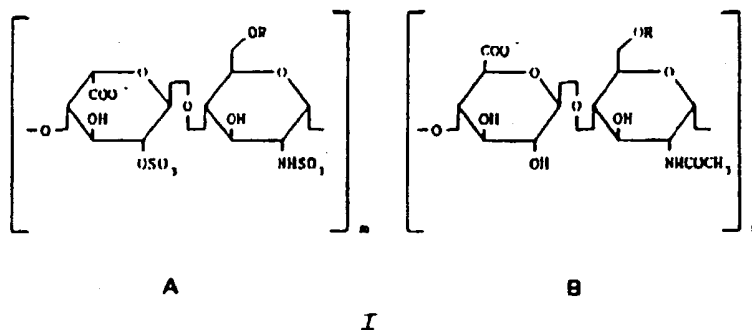
2-Aminoethylbisulfat, Taurin, Thioglykolsäure,  $\beta$ -Alaninethylester, L-Cystin, Hydroxylamin, Glycyltaurin, Cysteinyltaurin, Glycylcystein, Glycylphenylalanin, Glycyltyrosin, 2-Aminoethanol, Glycinester mit 2-Aminoethanol, Glycinamid mit 2-Aminoethanol, Arginyllysine, Arginin, Lysin, 2-Aminoethanolester mit Essigsäure, Salicylsäure, Methionin, Glycylprolin,  $\gamma$ -Aminobuttersäure, Lysylprolylarginin, Threonyllysylprolin, Threonyllysine, Prolylarginin, Lysylprolin, Cholin, 4-(3-Aminopropyl)-2-hydroxybenzoesäure und 4-(2-Aminoethyl)-2-hydroxybenzoesäure ableitet.

# Revendications

1. Procédé de synthèse de glycosaminoglycane semi-synthétiques possédant le motif de l'héparine ou de l'héparane, de formule générale (III) :



dans laquelle  $p + q = m$ , avec  $p$  différent de zéro, et  $m$  et  $n$  étant des nombres entiers dont la valeur est comprise entre et 100,  $R$  représente l'hydrogène ou le résidu sulfate ( $SO_3^-$ ), et  $-Z(R_2)R_1$  représente un radical nucléophile dans lequel  $Z$  représente l'oxygène, le soufre, ou l'azote,  $R_1$  représente un groupe alkyle ( $C_{1-12}$ ) linéaire ou ramifié, des radicaux amino, aromatiques, diazoïques, ou hydroxyle, substitués ou non substitués, et  $R_2$  est nul, ou représente l'hydrogène ou un radical alkyle ( $C_{1-6}$ ) linéaire ou ramifié, ou bien pris avec  $R_1$ , forme un cycle hétérocyclique, lequel procédé comprend la réaction d'un glycosaminoglycane possédant le motif de l'héparine ou de l'héparane, de formule générale (I) :



avec 1 à 200 équivalents molaires, par rapport à l'unité dimère de la glycosaminoglycane possédant le motif de l'héparine ou de l'héparane, de formule générale (II), d'un réactif nucléophile dont le radical est inclus dans la formule générale (II) :



en solution aqueuse en présence d'une quantité de base minérale ou organique suffisante pour salifier tout groupe

acide présent dans les réactifs nucléophiles, et/ou pour libérer les mêmes réactifs nucléophiles de tout sel qu'ils peuvent avoir avec des substances de nature acide, et pour créer un excès d'alcalinité tel que le mélange réactionnel passe de 0,01N à 1N par rapport à la base utilisée, éventuellement en atmosphère de gaz inerte, sous agitation pendant une période de temps comprise entre 24 et 96 heures à une température comprise entre 10°C et 40°C, la neutralisation du pH de la solution aqueuse froide, par addition d'une solution aqueuse d'acide chlorhydrique, éventuellement en éliminant l'excès de réactif nucléophile par extraction à l'aide d'un solvant non miscible à l'eau, ou par filtration, la soumission de la solution aqueuse à une dialyse avec de l'eau courante, et avec de l'eau distillée, et l'isolement du produit au moyen d'une lyophilisation de la solution aqueuse le contenant, ou par une précipitation par addition d'un solvant approprié.

2. Procédé selon la revendication 1, caractérisée par le fait que la quantité de réactif nucléophile est comprise entre 10 et 100 équivalents molaires par rapport à l'unité dimère du glycosaminoglycane de formule générale (I), et que la concentration dudit glycosaminoglycane est comprise entre 1 % et 5 %.
3. Procédé selon la revendication 1, caractérisé par le fait que la base utilisée est choisie parmi l'hydroxyde de sodium, l'hydroxyde de potassium, et la triéthylamine, et que l'excès d'alcalinité est tel que le mélange réactionnel passe de 0,5N à 1N par rapport à la base utilisée.
4. Procédé selon la revendication 1, caractérisé par le fait que le radical  $-Z(R_2)R_1$  dérive d'amines primaires ou secondaires, d'amines secondaires hétérocycliques, d'amino-alcools, d'aminothiols, d'acides aminés, d'amino-esters, de peptides, d'alcools, de phénols, de mercaptans, de dithiols, de thiophénols, d'hydroxylamine, d'hydrazines, d'hydrazides, et d'azoture de sodium.
5. Procédé selon la revendication 4, caractérisé par le fait que le radical  $-Z(R_2)R_1$  dérive de glycine, de glycyglycine, de L-cystéine, d'acétyl-L-cystéine, de L-cystéine éthyle ester, de 2-aminothiophénol, de 1,3-propanedithiol, de cystéamine, d'azoture de sodium, de 2-aminoéthyle bisulfate, de taurine, d'acide thioglycolique, de  $\beta$ -alanine éthyle ester, de L-cystine, d'hydroxylamine, de glycyLtaurine, de cystéinyLtaurine, de glycyLcystéine, de glycyLphénylalanine, de glycyLtyrosine, de 2-aminoéthanol, d'ester de glycine et de 2-aminoéthanol, d'amide de glycine et de 2-aminoéthanol, d'arginyLlysine, d'arginine, de lysine, d'ester de 2-aminoéthanol et d'acide acétique, d'acide salicylique, de méthionine, de glycyLproline, d'acide  $\gamma$ -aminobutyrique, de lysyLprolylarginine, de thréonyLlysylproline, de thréonyLlysine, de prolylarginine, de lysyLproline, de choline, d'acide 4-(3-aminopropyl)-2-hydroxybenzoïque, et d'acide 4-(2-aminoéthyl)-2-hydroxybenzoïque.